Exhibit A

#5

PATENT

Docket No. 240042052402

May 19, 01 Barry

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4/14/02 Date

Silvia Reaves

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Jeffrey S. Glenn

Serial No.: 09/028,655

Filing Date: February 24, 1998

For: METHOD FOR INHIBITION OF VIRAL MORPHOGENESIS

Examiner: B. Brumback

Group Art Unit: 1642

DECLARATION OF JEFFREY S. GLENN PURSUANT TO 37 C.F.R. §1.132

BOX AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Jeffrey S. Glenn, declare as follows:

1. I am the sole inventor of the subject matter claimed in the above-referenced application.

Considered 7-24-01 BB

2. I have conducted experiments demonstrating that:

1) CXXX boxes of several distinct viral proteins can serve as substrates for prenylation; and

2) FTI-277, a prenylation inhibitor, can effectively inhibit the production of HDV virions at a concentration that does not significantly affect general protein synthesis and secretion, and does not significantly affect overall cell metabolism.

These experimental results are set forth in the following paragraphs 3-12 and in attached Figures 1-4.

A. Prenylation of the other viral CXXX boxes

3. CXXX boxes from the proteins of several representative viruses were selected:

1) -CDLS is the CXXX box from the 3D protein (replication polymerase protein) of hepatitis A virus (HAV). The virus is known to replicate in association with cellular membranes. Therefore, prenylation of the replication protein is an ideal antiviral target;

2) -CTYV is the CXXX box from the UL32 protein of herpes simplex virus (HSV). There is genetic evidence showing that the UL32 protein is important for production of HSV virus particles. It is not required for replication of the viral genome, but rather appears to act in assembly of the virus. Prenylation of UL32 would be an ideal mechanism to anchor nascent particle assembly to the intracellular membrane sites where assembly occurs; and

3) -CRIQ is the CXXX box of TRL9 from cytomegalovirus (CMV). TRL9 was chosen as an example of an open reading frame encoded in a virus, but of completely unknown function. As such, there is no inherent bias in its selection attributable to any inferred role in its encoding virus' lifecycle. It can thus serve as a "generic" viral CXXX box.

The above-selected CXXX boxes are representative because HDV has a circular, negative-stranded RNA genome, HAV has a linear, plus-stranded RNA genome, and HSV and CMV have double-stranded DNA genomes.

4. These CXXX boxes were substituted for the native CXXX box of the large delta antigen using PCR mutagenesis and standard molecular cloning techniques. The following oligo pairs were used for the PCR mutagenesis:

1) For CDLS construct (hepatitis A virus):

5'-GGCTTCGTCCCCAGTCTGCAGGGAGTCCCGG-3' and

5'-GGGGCCGGATCCCGCTTTATTTACGAGAGGTCACAACTCTGGGG-3'.

2) For CTYV construct (herpes simplex virus):

5'-GGCTTCGTCCCCAGTCTGCAGGGAGTCCCGG-3' and

5'-GGGGCCGGATCCCGCTTTATTTACACGTATGTACAACTCTGGGG-3'.

3) For CRIQ construct (cytomegalovirus):

5'-GGCTTCGTCCCCAGTCTGCAGGGAGTCCCGG-3' and

5'-GGGGCCGGATCCCGCTTTATTTACTGGATTCGACAACTCTGGGG-3'.

5. The chimeric proteins were expressed in rabbit reticulocyte lysates, which are known to contain active prenyltransferases, in the presence of ^{3}H-mevalonate (the metabolic precursor of prenyl lipids), using the procedures described in Glenn et al, *Science*, 256:1331-1333 (1992). Also expressed in parallel were large delta antigen with its native CXXX box, as positive control, and small delta antigen which lacks a CXXX box, as negative control.

6. The expressed proteins were isolated by immunoprecipitation with an antibody specific for delta antigen, and analyzed by SDS-polyacrylamide gel electrophoresis followed by transfer to nitrocellulose membrane and fluorography, as described in Glenn et al, *Science*, 256:1331-1333 (1992).

7. The results show that the viral CXXX boxes from the three chimeric proteins can all be prenylated (Figure 1). As expected, the CXXX box from the native large delta antigen was also prenylated, and the small delta antigen which lacks a CXXX box and should therefore not be a substrate for prenylation indeed remained completely unmodified.

8. It is thus shown that CXXX boxes from different types of proteins ranging from well characterized to still unknown functions encoded in several quite distinct classes of viruses can be prenylated.

B. Inhibition of HDV virion production with FTI-277

9. Completely infectious HDV particles were produced using the system disclosed in Sureau et al., *J. Virol.*, 66:1241-5 (1992). Co-transfection of Huh-7 cells, a liver-derived cell line, with plasmids encoding the complete HDV and HBV genomes yielded HDV virions released into the media supernatant (Figure 2). Such released virions contain an intact HDV genome.

10. As shown in Figure 3, one week after the produced virions were inoculated onto cultures of primary human hepatocytes, at least 5-10% of the latter displayed the nuclear staining pattern characteristic of HDV infection when analyzed by immunofluorescence with an antibody against delta antigen. Thus, not only do the produced virions contain an intact RNA genome, but they are also infectious. This represents the first use of cultured human primary hepatocytes as a target for HDV infection.

11. FTI-277, a prenylation inhibitor, was tested for its ability to inhibit HDV virion production. As shown in Figure 4, while in the absence of drug virions were readily produced, they were dramatically inhibited at mid-nanomolar concentration of FTI-277. At micromolar concentrations of FTI-277, there were no detectable HDV virions produced. Non-specific toxicity was assessed by free HBV surface antigen assay, which assesses effects on general protein synthesis and secretion, and a standard XTT assay, which measures overall cell metabolism. As shown in Figure 4, FTI-277 can effectively inhibit HDV virion production at a concentration that essentially does not affect general protein synthesis and overall cell metabolism.

12. Taken together, the above results demonstrate that pharmacological inhibition of prenylation can interfere with virus particle production. Furthermore, compounds like FTI-277, which inhibit prenylation, represent a novel class of antiviral agents.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to by true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Palo Alto, California, on April 13, 2000.

Jeffrey S. Glenn

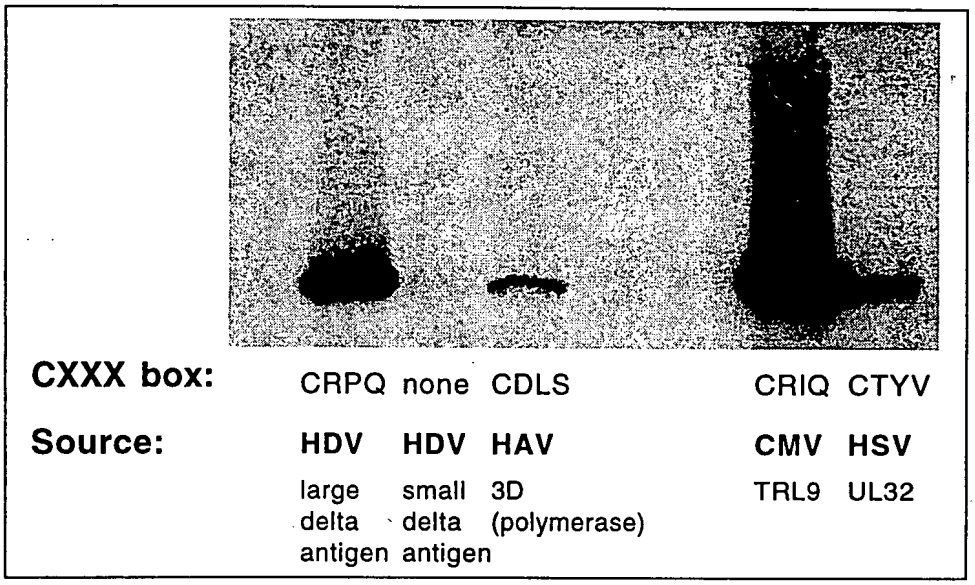

Figure 1. CXXX boxes from a variety of viruses are subject to prenylation. CXXX boxes located in the proteins of several different types of viruses were selected and used to replace the normal CXXX box of HDV's large delta antigen. The resulting chimeric proteins were then synthesized and tested for their ability to undergo prenylation in a rabbit reticulocyte lysate system, as previouly described in Glenn et al. Science, 256:1331-1333 (1992). Large delta antigen (which has a CXXX box capable of undergoing prenylation) and small delta antigen (which does not have a CXXX box capable of undergoing prenylation) served as positive and negative controls, respectively. Note CXXX boxes found in hepatitis A virus (HAV), cytomegalovirus (CMV) and herpes simplex virus (HSV) all undergo prenylation.

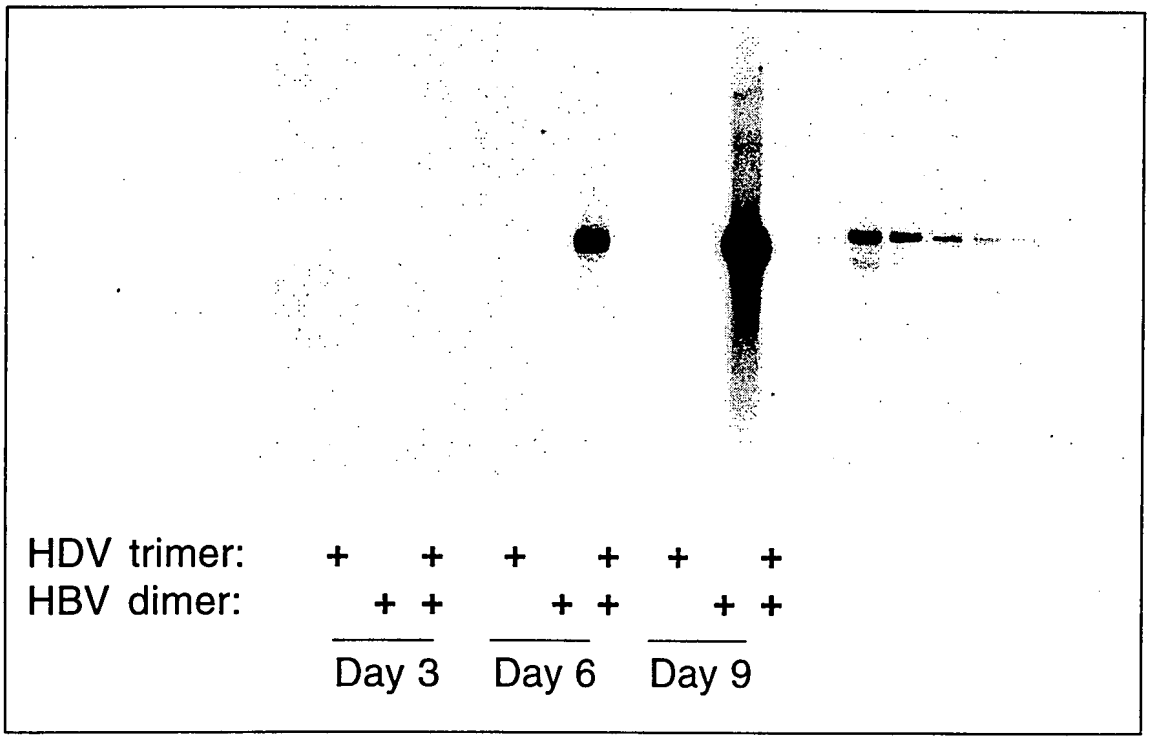

Figure 2. Production of HDV genome-containing virions. Huh-7 cells were transfected with plasmids encoding the HDV genome and the HBV genome, or with either plasmid alone. Media supernatants collected on the indicated days after transfection were then analyzed by northern blot with a probe for HDV genomic RNA to detect the presence of genome-containing virions. In vitro transcribed linear HDV RNAs (a small fraction of which have undergone autocatalytic processing at the genomic strand ribozyme site) were included on the right side of the blot as standards. Note that the HDV RNA in the virions migrates slightly faster than the linear standards, as is characteristic for circularized genomic RNA contained in intact virions.

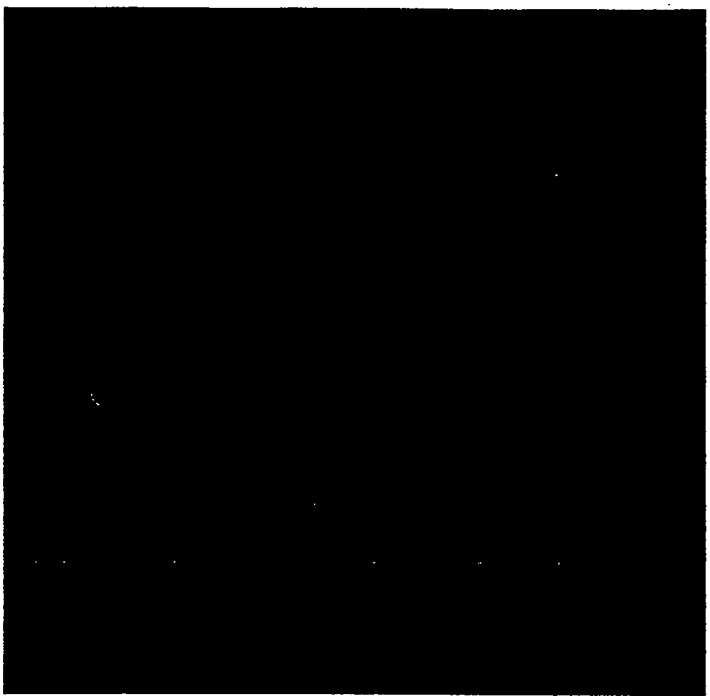

Figure 3. Infection of human primary heptocytes with HDV. Primary human hepatocytes were inoculated with produced HDV particles, cultured for one week, fixed, and stained with a human anti-delta antigen serum as primary antibody and rhodamine-labelled goat anti-human reagent as secondary antibody. Note characteristic nuclear staining pattern (in red) of delta antigen in several hepatocytes.

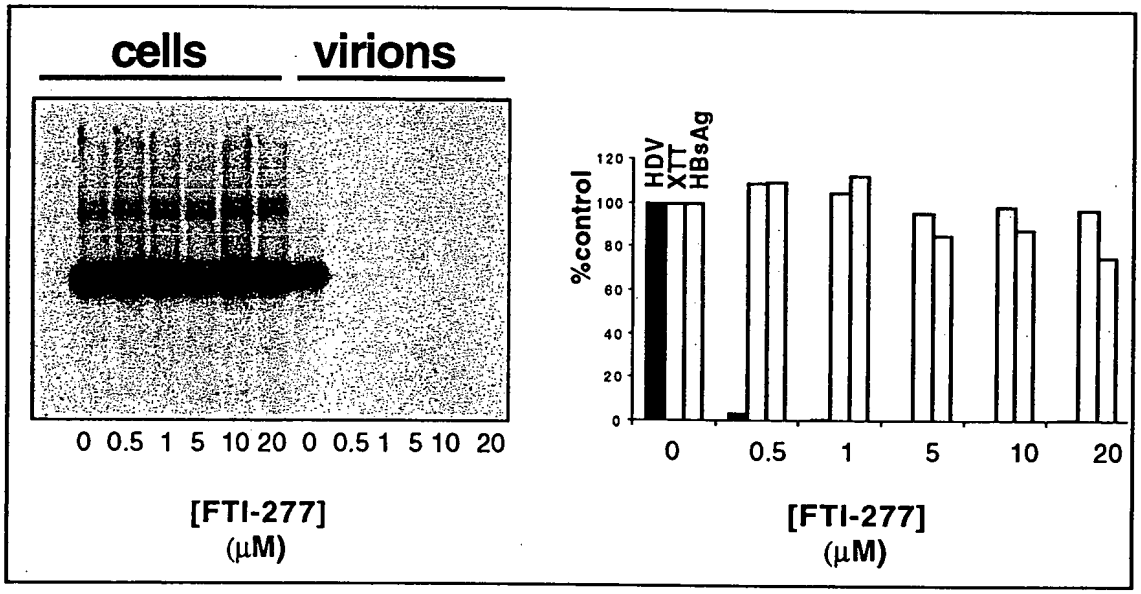

Figure 4. FT1-277 inhibits production of HDV virions. Huh-7 cells were co-transfected with HDV and HBV encoding plasmids to establish production of HDV virions, as described in the text, and grown in the presence of the indicated concentrations of FTI-277. HDV genome replication in the cells, and the amount of HDV genome-containing virions released into the supernatants, were monitored by northern blot analysis with a probe for HDV genomic RNA (left panel). The results were quantitated with a phosphoimager and the amount of virions produced at each concentration of FTI-277, expressed as a percentage of the no drug control, was plotted (purple bars, right panel). Also plotted are the results of assays for cellular metabolism (XTT assay, yellow bars), and general protein synthesis and secretion (HBV surface antigen released into the media supernatants, blue bars).